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Short communication

Cyclic amide derivatives as potential prodrugs. Synthesis and evaluation of *N*-hydroxymethylphthalimide esters of some non-steroidal anti-inflammatory carboxylic acid drugs

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Abstract – *N*-Hydroxymethylphthalimide (HMPhI) esters **5a–d** of some nonsteroidal anti-inflammatory drugs were synthesized and evaluated as potential prodrugs with the aim of depressing the gastrotoxicity of the parent drugs by temporarily masking the carboxylic acid function. The ester prodrugs were synthesized through condensation of *N* -hydroxymethylphthalimide and the mixed carboxylic-carbonic anhydride intermediate or the corresponding acid chloride of the parent acid. Their structures were confirmed by ^{1}H-NMR spectra and the purity has been assessed by TLC and elemental analyses. An HPLC method has been developed for investigation of the hydrolysis kinetics in aqueous buffer solutions and in 80% rabbit plasma. The lipophilicity parameters log *P* and log *k'* were determined and showed that the prodrugs were found to be more lipophilic (log $P > 2$) than the parent drugs. A considerable chemical stability of all compounds ($t_{1/2}$ = 4.7–21.9 h) has been observed in non-enzymatic simulated gastric fluid (hydrochloric acid buffer of pH 1.3), while at pH 7.4 only prodrugs **5b–d** are sufficiently stable ($t_{1/2}$ ~ 3–5 h). Meanwhile, rapid conversion to the parent drugs **3a–d** was observed in 80% rabbit plasma ($t_{1/2}$ ~ 1.0–11.5 min). Potential ulcerogenicity on rat stomach mucosa of the prodrugs and the parent drugs after oral administration for 4 days was studied using a scanning electron microscope. Gross observations and scanning electro-micrographs showed that the prodrugs are significantly less irritating to gastric mucosa than the parent NSAIDs. This result suggests that *N*-hydroxymethylphthalimide esters may be useful as nonulcerogenic prodrug forms for acidic NSAIDs. © Elsevier, Paris

NSAI prodrugs / *N*-hydroxymethylphthalimide / lipophilicity / chemical and enzymatic stability / ulcerogenicity

1. Introduction

The gastrointestinal side effects of NSAIDs are generally attributed to direct and/or indirect mechanisms [1–4]. The direct contact effect results usually from a local irritation produced by the acidic group of the NSAID and local inhibition of prostaglandin synthesis in the GI tract. The indirect mechanism is due to a generalized systematic action occurring after absorption and can be demonstrated following intravenous dosing [4, 5].

A possible approach to solve these delivery problems may be derivatization of the carboxylic function of the NSAIDs to produce prodrug forms with adequate stability at the acidic pH of the stomach. Thus, such derivatization may, on one hand, prevent the local irritation on stomach mucosa and, on the other hand, be capable of releasing the parent drug spontaneously or enzymatically in the blood following their absorption. The utility of amides as pro-moiety in the design of prodrugs of carboxylic acids has been recently reported [6–9]. The tertiary *N*-acyloxymethylamides **1** are the most prevailing prodrug forms, owing to their significant stability at pH 7.4 [9, 10].

The present work reports on the application of *N*-hydroxymethylimides **2** as pro-moiety for ester prodrugs of some acidic NSAIDs. The carbonyl groups of the imide moiety may be regarded as isosteric with the methylene groups in the corresponding tertiary amides. Replacing the *N,N*-disubstituted amide group with an imide moiety might represent a good fit for the trimethyl binding site of the nonspecific esterases [11, 12], resulting in higher bioconversion rates. This study describes the synthesis, physicochemical properties, kinetics for chemical and enzymatic hydrolysis of *N*-hydroxymethylphthalimide esters of aspirin, ibuprofen, naproxen and indomethacin for potential use as prodrugs for oral delivery.

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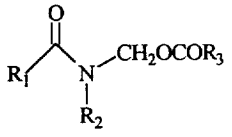

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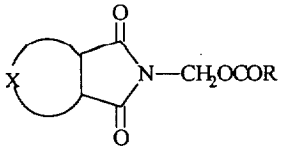

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2. Chemistry

The synthesis of the ester prodrugs **5a–d** (*figure 1*) was achieved by interaction of the mixed carboxylic-carbonic anhydride intermediate, resulting from the respective carboxylic acids **3a–c** and ethyl chloroformate in the presence of triethylamine, or the acid chloride of indomethacin **3d** with the pro-moiety *N*-hydroxymethylphthalimide (HMPhI) **4**. The structures of synthesized compounds **4** and **5a–d** were confirmed by ^{1}H-NMR spectroscopy. The purity was determined by elemental analysis, TLC and HPLC. As expected, the esterification results in a downfield shift (~ 1.0 ppm) of the signal corresponding to the methylenic protons relative to its position in the spectrum of *N*-hydroxymethyl phthalimide (δ = 5.1 ppm). Meanwhile, the aromatic protons of the isoindole-1,3-dione moiety were not affected by esterification and appeared as multiplet at ~ 8.0 ppm. The results of elemental analyses (C, H and N) of the synthesized compounds were in all cases within ± 0.4% of the theoretical values.

3. Results and discussion

3.1. Lipophilicity of the prodrugs

It is generally accepted that good absorption of orally administered drugs could be attained when the value of the octanol/water partition coefficient ≥ 100 or more (log $P \geq 2$) [13]. To assess this potential, the apparent partition coefficients (log P) of the studied ester prodrugs **5a–d** and the parent drugs **3a–d** were determined in an *n*-octanol/phosphate buffer of pH 7.4. The concentration of the compounds in octanol and buffer layers were determined by least-square

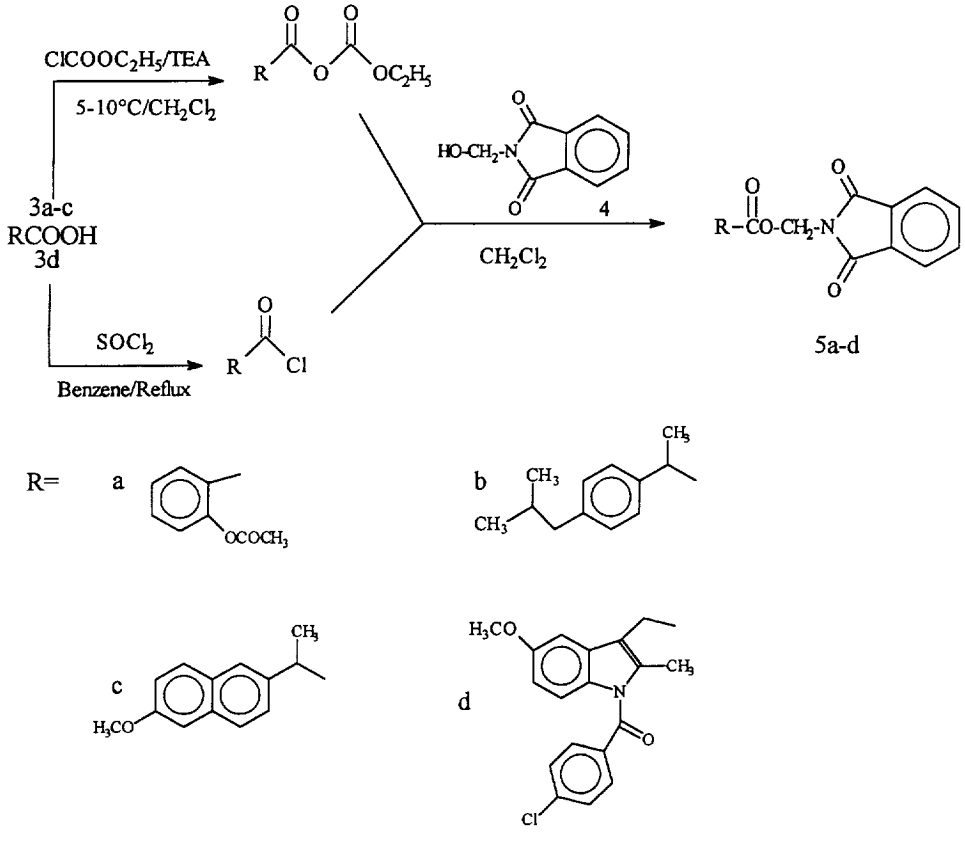

Figure 1. Synthetic pathway for the preparation of the prodrugs **5a–d**.

Table I. Chromatographic data for analytical HPLC[a] of the NSAIDs **3a–d** and the prodrugs **5a–d**.

Compound	Eluent	Detection λ_{max} (nm)	RT (min)	Log P[b]	Log k'[c]
3a	MeOH/phosphate buffer; pH 4 (70:30)	270	3.0	1.42	– 0.865
3b	MeOH/H_2O (90:10)	228	3.8	3.73	0.247
3c	MeOH/H_2O (80:20)	232	3.7	3.00	– 0.130
3d	MeOH/H_2O (90:10)	257	3.1	2.33	–0.018
4	ACN/H_2O/TFA (35:65:0.1)	230	3.7	–	–
5a	MeOH/phosphate buffer; pH 4 (70:30)	270	5.9	3.23	– 0.091
5b	MeOH/H_2O (90:10)	228	4.7	5.36	0.612
5c	MeOH/H_2O (80:20)	232	5.1	5.03	0.249
5d	MeOH/H_2O (90:10)	257	6.8	5.64	0.391
6	ACN/H_2O/TFA (35:65:0.1)	230	4.1	–	–

[a]Flow rate = 1 mL/min; [b]in octanol/phosphate buffer pH 7.4; [c]eluent methanol/phosphate buffer pH 4.0.

equations, derived by correlating the peak areas in HPLC to a known concentration of each compound.

The chromatographic lipophilicity parameters (log k') were also determined by means of the RP-HPLC capacity factor (k') in a methanol/phosphate buffer (80:20) of pH 4.0 as mobile phase. The determined values of log P and log k' are listed in *table I*, indicating that all prodrugs possess optimum lipophilicities (log P ~ 2–5) required for oral absorption. As previously observed, a linear relationship might exist between the lipophilicity parameters log k' and log P [14]. Regression analysis of the determined log P and log k' values of the parent NSAIDs **3a–d** and their prodrugs **5a–d** gives:

$$\log P = 3.569 + 3.016\ (\pm 0.622) \log k', \qquad (1)$$

$$n = 8,\ r = 0.891,\ \mathrm{SE} = 0.795,\ F = 23.2,\ P = 0.003.$$

Exclusion of the deviating values of indomethacin **3d** results in a better linear correlation between log k' and log P, (eq. (2)). This relationship would likely allow for a calculation of log P for related compounds in this series:

$$\log P = 3.742 + 2.951\ (\pm 0.492) \log k', \qquad (2)$$

$$n = 7,\ r = 0.937,\ \mathrm{SE} = 0.582,\ F = 36.0,\ P = 0.001.$$

3.2. Kinetics of hydrolysis

The kinetics of chemical and enzymatic hydrolysis of the HMPhI ester prodrugs **5a–d** were studied at 37 °C in aqueous buffer solutions of pH 1.3 and pH 7.4 as well as in 80% rabbit plasma. The reactions were monitored by HPLC for the decrease in ester concentration vs. time and were found to display pseudo-first-order kinetics over several half-lives. The rate constant (k_{obs}) for hydrolysis and the corresponding half-life of each of the studied prodrugs are listed in *table II*. *Figures 2*, *3* and *4* illustrate plots of pseudo-first-order kinetics for hydrolysis of the ester prodrugs **5a–d** in hydrochloric acid buffer of pH 1.3, phosphate bufer of pH 7.4 and in 80% rabbit plasma respectively.

The rate data in *table II* show that all the studied prodrugs are stable in aqueous buffer solution of pH 1.3 ($t_{1/2}$ ~ 5–22 h). This acid stability implies that the compounds pass unhydrolyzed through the stomach on oral administration. At pH 7.4 only the prodrugs **5b–d** are adequately stable ($t_{1/2}$ ~ 3–5 h) to be absorbed intact from the intestine (pH ~ 7.0). The prodrug of aspirin **5a** undergoes rapid decomposition at pH 7.4 ($t_{1/2}$ ~ 8 min). This might be attributed to the mutual activation of the *ortho*-positioned acetoxy and HMPhI ester groups. Further investigations are currently undertaken to assign the respective half-life for each of the two competing reactions. As has been reported

Table II. Kinetic data for chemical and enzymatic hydrolysis of the prodrugs **5a–d**.

Compound	pH 1.3[a]		pH 7. 4[b]		Plasma[c]	
	K_{obs} (h^{-1})	$t_{1/2}$ (h)	K_{obs} (h^{-1})	$t_{1/2}$ (h)	K_{obs} (min)	$t_{1/2}$ (min)
5a	0.1589	4.4	5.322	0.13	–	< 1.0
5b	0.0991	6.99	0.1736	3.99	0.1338	5.2
5c	0.0786	8.8	0.2545	2.8	0.0757	9.2
5d	0.0317	21.9	0.1368	5.1	0.0602	11.5

[a]Hydrochloric acid buffer (0.2 M); [b]phosphate buffer (0.02 M); [c]80% rabbit plasma.

by Bundgaard et al. [7], a prerequisite for any true aspirin prodrug is that the masking group should be acid-stable to prevent direct contact effects of aspirin on stomach mucosa. The prepared HMPhI ester prodrug of aspirin **5a** fulfills this requirement, since it showed good stability at acidic pH ($t_{1/2}$ = 4.7 h).

In 80% rabbit plasma the hydrolysis of the ester prodrugs **5a–d** proceeds much faster than in aqueous buffers. Comparison of the rate data in *table II* indicates that the values of k_{obs} are greater in 80% rabbit plasma than the corresponding values in aqueous buffer of pH 7.4 by factors in the range ~ 15–50. This confirms the assumption that the imide moiety might afford a good fit for the trimethylamino site of the esterases in plasma. It should also be noted that variations in chemical and enzymatic rates of hydrolysis of the prodrugs **5a–d** might be attributed to the structural differences of the carboxylic acids moiety. The enzymatic reactivity appears to be affected by steric factors relating to the size of the acyl moiety. Prodrug **5a** with smaller acyl structure enables facilitated access to the active site of the hydrolytic enzyme, so

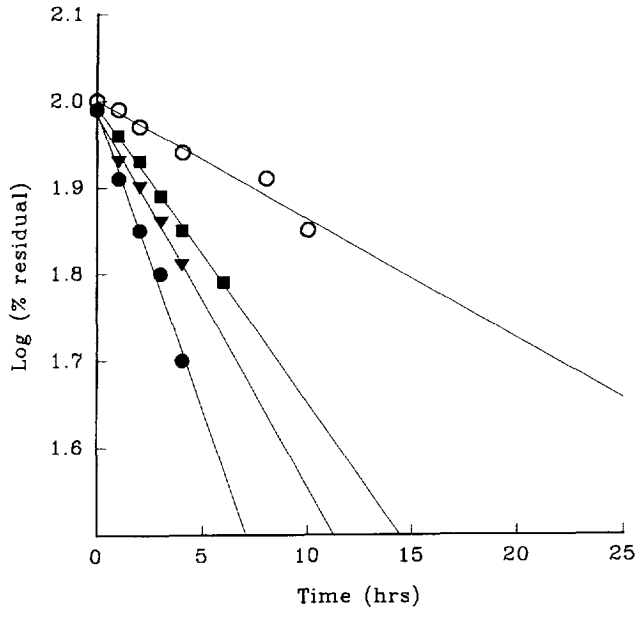

Figure 2. First-order kinetic plots for hydrolysis of prodrugs **5a–d** in hydrochloric acid buffer of pH 1.3 at 37 °C. ●: **5a**; ▼: **5b**; ■: **5c**; ○: **5d**.

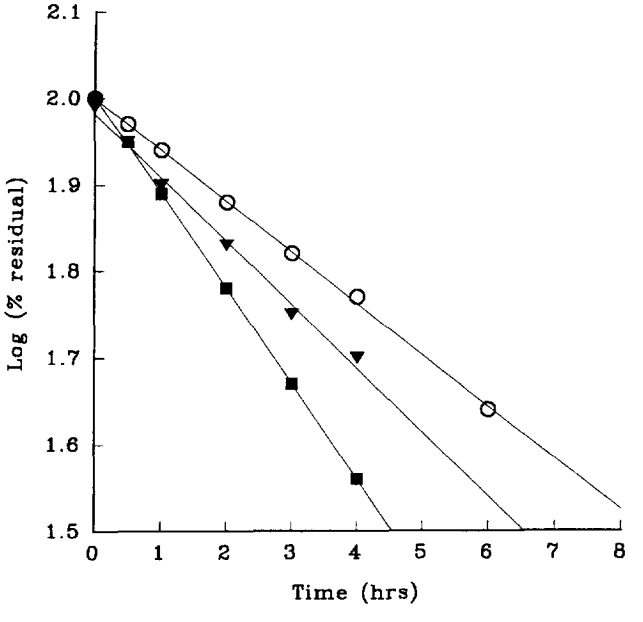

Figure 3. First-order kinetic plots for hydrolysis of prodrugs **5b–d** in 0.02 M phosphate buffer of pH 7.4 at 37 °C. ▼: **5b**; ■: **5c**; ○: **5d**.

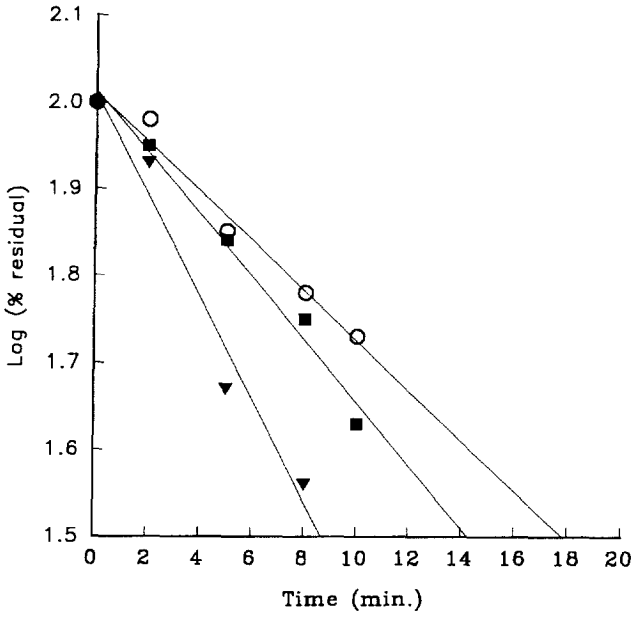

Figure 4. First-order kinetic plots for hydrolysis of prodrugs **5b–d** in 80% rabbit plasma at 37 °C. ▼: **5b**; ■: **5c**; ○: **5d**.

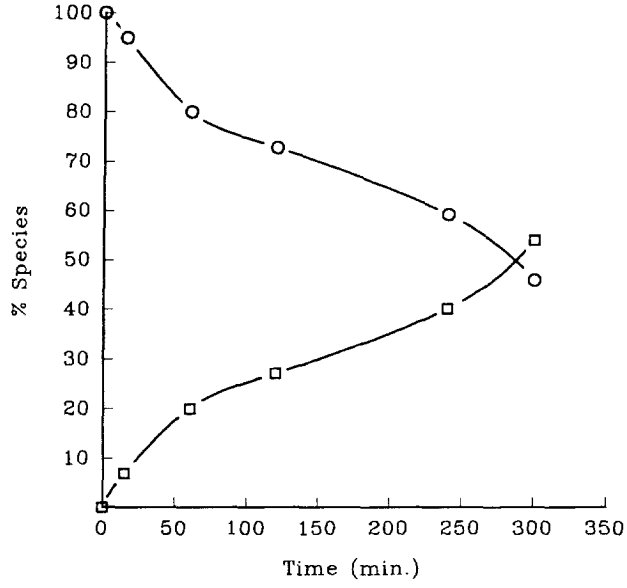

Figure 6. Time courses for disappearance of **5d** (○) and appearance of **3d** (□) during hydrolysis in 0.02 M phosphate buffer of pH 7.4 at 37 °C.

that no ester peak could be detected in HPLC after one minute of incubation with 80% rabbit plasma. In the case of prodrug **5d**, the *p*-chlorobenzoyl substituent retarded the proper fitting to the active sites of the enzyme ($t_{1/2}$ = 11.5 min).

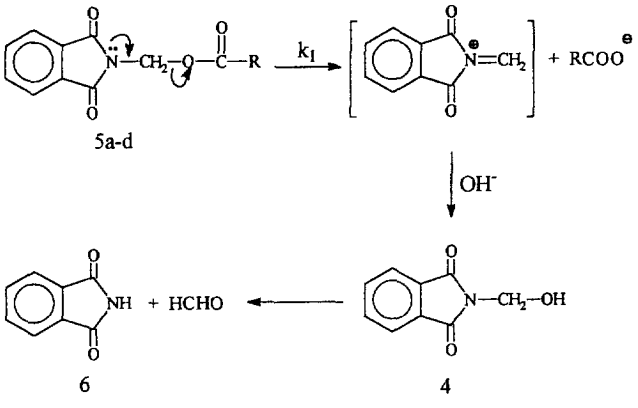

Figure 5. Suggested mechanism for hydrolysis of the prodrugs **5a–d**.

The ultimate products of the decomposition of the prodrugs **5a–d**, under chemical or enzymatic conditions, are the parent NSAID and phthalimide **6** as depicted in *figure 5*. This was shown by HPLC analysis of the reaction solutions, using a solvent system affording separation of the esters **5a–d**, the parent acids **3a–d** and phthalimide **6**. An example of product analysis of the hydrolysis of the prodrug **5d** at pH 7.4 is shown in *figure 6*, indicating subsequent formation of indomethacin **3d**.

As previously stated for structurally related prodrug forms [15], the cleavage is expected to proceed in a two-step-reaction. The relative high reactivity of the prodrugs at neutral pH ($t_{1/2}$ ~ 0.1–5 h) is indicative of the involvement of another mechanism of hydrolysis other than the nucleophilic S_N2 reaction normally occurring in ester hydrolysis. The most likely mechanism involved is suggested to be a unimolecular elimination–addition process, which is regarded as an S_N1 reaction with the formation of a transient *N*-acyliminium ion intermediate (*figure 5*). The rate-determining step (k_1) is the elimination of the carboxylate anion to give an iminium ion, which in a subsequent fast step is attacked by hydroxide ions,

giving *N*-hydroxymethylphthalimide **4**. This assumption is in accordance with previous observations stated for similar compounds [8, 16].

3.3. Gastrointestinal tolerance

The ulcerogenic liability of the prepared prodrugs **5a–d** was tested in comparison to the parent NSAIDs **3a–d** following oral administration for 4 days in rats. Gross observation of stomachs revealed obvious widespread haemorrhagic spots in the drugs-treated groups. In our laboratory we have already introduced the utility of scanning electron-micrographs as a highly precise and self-explanatory tool for the investigation of ulcerogenicity of NSAI agents [17]. *Figure 7* shows side-by-side scanning electron-micrographs for specimen stomachs of drugs-treated and prodrugs-treated rats. As can be seen from *figure 7* (A), (C), (E) and (G) the prodrugs-treated groups show intact mucosal layers and were to a high extent identical with the group receiving only the vehicle (*figure 7* (I)). The drugs-treated groups, *figure 7* (B), (D), (F) and (H), are characterized by mucosal damage in addition to severe ulcerations of the submucosal layer in some cases. These findings indicate that the prodrugs **5a–d** are significantly less irritating to the gastric mucosa than the parent drugs **3a–d**.

4. Conclusion

In vitro and in vivo evaluations indicated that the *N*-hydroxymethylphthalimide esters **5b–d** have promising properties as prodrugs for oral delivery of the studied NSAIDs. They are more lipophilic, have adequate acid stability with higher gastrointestinal tolerance and get rapidly cleaved to the parent drugs in 80% rabbit plasma. However, the HMPhI-ester of aspirin **5a** is found to be too relatively unstable at neutral pH value to be considered as a useful prodrug. Further trials are undertaken for optimization and development of a carrier system with satisfactory physicochemical properties.

5. Experimental protocols

5.1. Chemistry

Non-steroidal antiinflammatory drugs **3a–d** were provided by various pharmaceutical companies in Cairo and used as received. All other chemicals and reagents used in the syntheses were reagent-grade and those for the kinetic studies were of analytical grade. Fresh doubly distilled water from all-glass apparatus was used in the preparation of all the solutions. Melting points were determined on an electrothermal melting point apparatus (Stuart Scientific, England) and were uncorrected. Precoated silica gel plates (Kiesel gel 60G F254 nm, Merk, Germany) were used for TLC. ^{1}H-NMR spectra were recorded on a Varian EM-360L NMR spectrometer (Varian, USA), TMS was used as internal standard and chemical shifts are given in ppm. Microanalyses were carried out on a Perkin-Elmer 240 °C elemental analyzer, Faculty of Science, Assiut University.

5.1.1. Synthesis of N-hydroxymethylphthalimide 4

A suspension of phthalimide 14.7 g (0.1 mol) and 5.5 mL formaldehyde solution (40%) in water (50 mL) was refluxed until a clear solution resulted. The hot solution was filtered and cooled overnight and the white crystalline product obtained was filtered and dried. Recrystallization from water gave 14.2 g (80%) of *N*-hydroxymethylphthalimide **4**: mp 140–141 °C, lit. [18] mp 138–141 °C; ^{1}H-NMR, DMSO-d_6, δ_H: 5.1 (2H, d, J = 7 Hz, N–CH_2–), 5.9 (1H, t, J = 7 Hz, –CH_2OH), 7.9 (4H, m, phthalimide–H). Found: C, 61.38; H, 3.91; N, 7.63. Calc. for $C_9H_7NO_3$: C, 61.02; H, 3.98; N, 7.91.

5.1.2. Synthesis of N-hydroxymethylphthalimide ester prodrugs 5a–c

Ethyl chloroformate 1.1 g (0.01 mol) was added dropwise to a cooled solution of the respective carboxylic acid **3a–c** (0.01 mol) and triethylamine 1.02 g (0.01 mol) in methylene chloride (50 mL), the mixture was stirred for further 30 min at 5–10 °C. *N*-hydroxymethylphthalimide 1.77 g (0.01 mol) was added over a period of 30 min and stirring was continued overnight at room temperature. The reaction mixture was then washed with water and 5% sodium hydrogen carbonate solution, dried over anhydrous sodium sulphate and filtered. The solvent was removed under reduced pressure and the residue recrystallized from the appropriate solvent. The following are new compounds:

2-[[(1,3-Dioxo-2,3-dihydro-1H-2-isoindolyl)methyl]oxycarbonyl]phenyl acetate ***5a***. 2.5 g (75%); mp 119–120 °C (acetone/petroleum ether); ^{1}H-NMR, $CDCl_3$, δ_H: 2.3 (3H, s, $COCH_3$), 6.0 (2H, s, –NCH_2O–), 7.3 (4H, m, Ph), 7.9 (4H, m, phthalimide–H). Found: C, 63.36; H, 3.91; N, 3.82. Calc. for $C_{18}H_{13}NO_6$: C, 63.72; H, 3.86; N, 4.13.

(1,3-Dioxo-2,3-dihydro-1H-2-isoindolyl)methyl 2-(4-isobutylphenyl)propionate ***5b***. 3.1 g (85%); mp 102–105 C (petroleum ether); ^{1}H-NMR, $CDCl_3$, δ_H: 1.0 [6H, d, J = 7 Hz, –CH_3)$_2$], 1.5 (3H, d, J = 7 Hz, – $CHCH_3$), 1.9 [1H, m, –$CH(CH_3)_2$], 2.4 (2H, d, J = 7 Hz, –$CHCH_2$–), 3.8 (1H, q, J = 7 Hz, –$CHCH_3$), 5.7 (2H, s, –NCH_2O–), 7.0 (4H, bs, Ph–H), 7.9 (4H, m, phthalimide–H). Found: C, 72.03; H, 6.30; N, 3.51. Calc. for $C_{22}H_{23}NO_4$: C, 72.31; H, 6.35; N, 3.83. This compound has been previously described without published characterization by Groutas [19].

(1,3-Dioxo-2,3-dihydro-1H-2-isoindolyl)methyl 2-(6-methoxy-1-naphthyl) propionate ***5c***. 3.1 g (80%); mp 152–153 °C (ethanol); ^{1}H-NMR, $CDCl_3$, δ_H: 1.6 (3H, d, J = 7 Hz, $CHCH_3$), 3.8 (1H, q, J = 7 Hz, –$CHCH_3$), 3.9 (3H, s, OCH_3), 5.9 (2H, s, –NCH_2O–), 7.2 (6H, m, naphthyl–H), 7.8 (4H, m, phthalimide–H). Found: C, 71.12; H, 4.68; N, 3.86. Calc. for $C_{23}H_{19}NO_5$: C, 70.94; H, 4.68; N, 3.59.

5.1.3. Synthesis of (1,3-dioxo-2,3-dihydro-1H-2-isoindolyl)-methyl 2-[1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1H-3-indolyl]acetate ***5d***

Thionyl chloride 3.84 g (0.03 mol) was added dropwise to a stirred suspension of Indomethacin 3.57 g (0.01 mol) in dried benzene (150 mL) and the mixture was refluxed for 3 h.

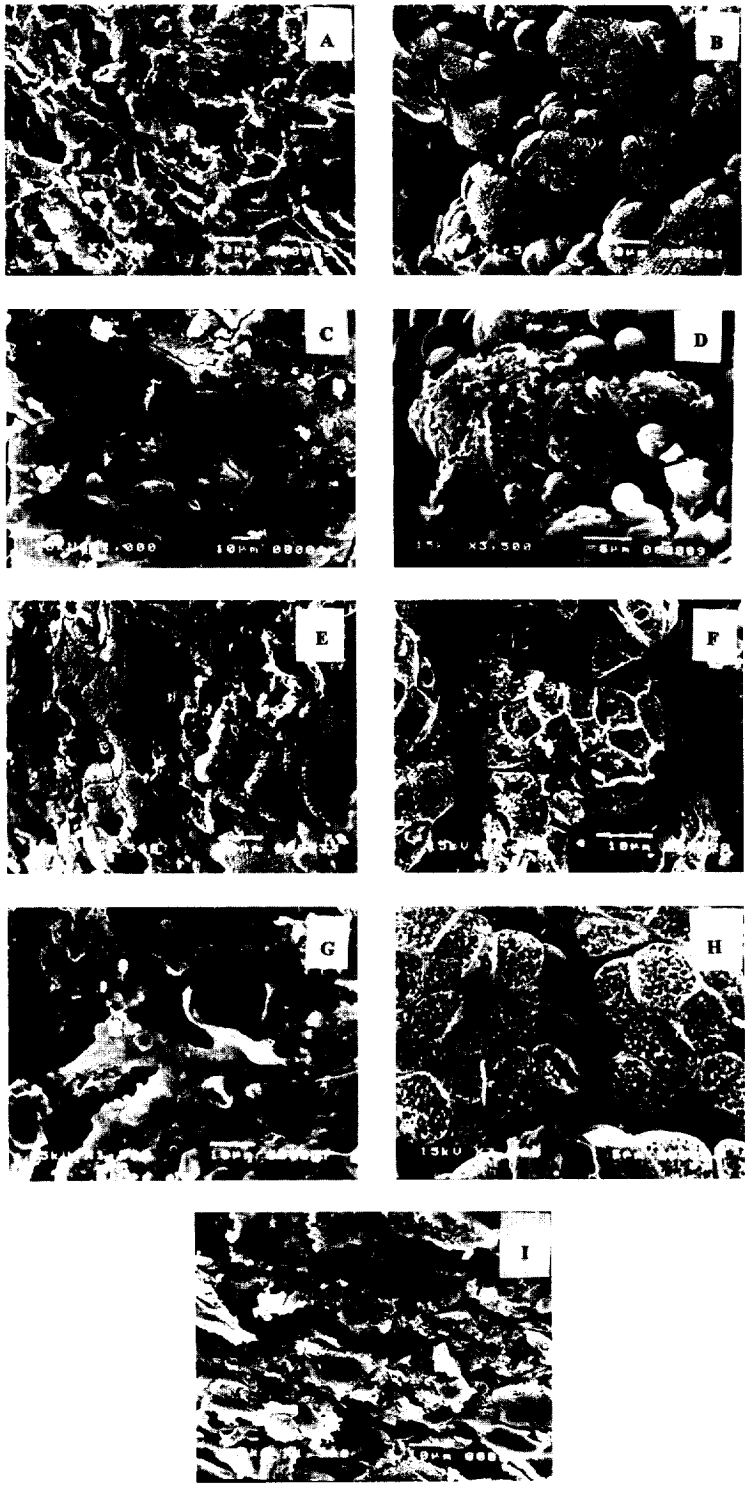

Figure 7. Scanning electromicrographs of rat stomachs following chronic dose administration, 4 days. A: **5a**; B: **3a**; C: **5b**; D: **3b**; E: **5c**; F: **3c**; G: **5d**; H: **3d**; I: control.

The solvent and excess thionyl chloride were removed under reduced pressure and the resulting acid chloride was dissolved in methylene chloride (50 mL) and added dropwise to a cooled solution containing *N*-hydroxymethylphthalimide 1.8 g (0.01 mol), triethylamine 1.1 mL (0.01 mol) and 4-dimethylaminopyridine 0.01 g in methylene chloride (50 mL). The reaction mixture was stirred for 10 h at room temperature, and the precipitated product was then filtered, dried and recrystallized from acetone. Yield 3.5 g (65%); mp 163–165 °C; ^{1}H-NMR, DMSO-d_6, δ_H: 2.3 (3H, s, $-CH_3$), 3.4 (2H, s, CH_2COO-), 3.9 (3H, s, $-OCH_3$), 5.7 (2H, s, $-NCH_2O-$), 7.0 (3H, m, indolyl–H), 7.7 (4H, bs, *p*-ClC_6H_4CO-), 8.0 (4H, m, phthalimide–H). Found: C, 65.51; H, 4.36; N, 5.54. Calc. for $C_{26}H_{21}ClN_2O_6$: C, 65.11; H, 4.11; N, 5.42.

5.2. Analytical method

The ester prodrugs **5a–d** and the respective NSAIDs **3a–d** were determined by reversed-phase HPLC procedures using a Knauer equipment (Fa. Knauer, Berlin, Germany) consisting of a Model 64 pump, a Model V 0890 Knauer tunable UV detector, a Shimadzu CR6A chromatopac data module integrator and a 20 µL loop injection valve. The column used is a reversed-phase Hypersil BDS C18 (250 x 4.6 mm; 5 µm particles) in conjunction with a cartridge guard precolumn. The chromatographic conditions were as summarized in *table I*. Least-squares equations, derived by correlating the recorded peak areas to the known concentrations for each compound were then used for calculation of the concentration in the studied samples.

5.3. Determination of lipophilicity parameters

The apparent partition coefficients log *P* of the prodrugs and the parent drugs were determined by dissolving the respective compound in octanol (2 mL) in 10 mL screw-capped test tubes followed by addition of an equal volume of phosphate buffer solution of pH 7.4. The two phases were then vortexed for 10 min and the tubes were centrifuged at 6000 rpm for 10 min. The layers were separated and diluted so that the final concentration of the prodrug was 1 x 10^{-5} M. Aliquots of 20 µL of each were then withdrawn, injected into the HPLC column and the correponding peak areas were recorded. All experiments were conducted in triplicate and the mean values were taken.

The chromatographic lipophilicity parameters (log *k'*) for the prodrugs **5a–d** and the parent drugs **3a–d** were also evaluated on the basis of the reversed-phase HPLC capacity factor using a methanol/phosphate buffer (80:20), pH 4.0, as eluent. In this method, the capacity factor (*k'*) of a solute was taken as a measure of the relative lipophilicity and calculated according to the following formula:

$$k' = (t_R - t_0)/t_0$$

where t_R is the retention time of the solute and t_0 denotes the elution time of the solvent. The results are listed in *table I*.

5.4. Kinetics of hydrolysis in aqueous solutions

A hydrochloric acid buffer of pH 1.3 as non-enzymatic simulated gastric fluid (SGF) and a 0.02 M phosphate buffer of pH 7.4 were used in this study. Reactions were initiated by adding 1 mL of a 10^{-4} M stock solutions (in dioxane) of the respective ester prodrug to 10 mL of the appropriate thermostated (37 ± 0.5 °C) aqueous buffer solution, containing 20% dioxane, in screw-capped test tubes. At appropriate time intervals samples of 20 µL were withdrawn and analysed by HPLC. Pseudo-first-order rate constants (k_{obs}) for the hydrolysis of the ester prodrugs were then calculated from the slopes of the linear plots of log (% residual prodrug) vs. time. The experiments were run in triplicate for each ester and the mean values of the rate constants were calculated.

5.5. Kinetics of hydrolysis in rabbit plasma

The reactions were initiated by adding 50 µL of 10^{-4} M stock solution of the ester prodrugs **5a–d** to 2.0 mL of the preheated 80% rabbit plasma and kept in a water bath at 37 ± 0.5 °C. At appropriate time intervals samples of 100 µL were withdrawn and added to 1 mL acetonitrile for deproteinization. After mixing and centrifugation at 10^4 rpm for 10 min, 20 µL were withdrawn and analyzed by HPLC for the remaining ester prodrug. Pseudo-first-order rate constants (k_{obs}) for hydrolysis were determined from the slopes of the linear plots of the log (% residual prodrug) vs. time. Triplicate experiments were carried out and the mean values of the rate constants were calculated.

5.6. In vivo ulcerogenicity study

A JEOL, JSM-5400LV scanning electron microscope was used for carrying out the scanning micrographs of rat stomach specimen at the Electron Microscope Unit, Assiut University. Groups of 6 male Wister rats (180–200 g) were fastened for 24 h and then administered a daily oral dose of 1 mL suspension of the tested ester prodrugs **5a–d** and their parent carboxylic acid drugs **3a–d** in 0.5% methylcellulose solution for four successive days. Doses were equivalent to 100 mg/kg of **3a**; 150 mg/kg of **3b**; 40 mg/kg of **3c** and 6.75 mg/kg of **3d** or the equivalent amounts of the respective prodrugs **5a–d**. The control group recieved an equal volume of the dispersion medium. All groups were denied access to food throughout this period and 24 h after the last dose the rats were sacrificed, so that the stomach could be removed, opened along the greater curvature and cleaned gently by dipping in saline, examined under a binocular magnifier for possible local ulcerations and the specimens were prepared for scanning in an electron microscope.

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